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The Effect of Multi-Strain Probiotics Formula on Blood Pressure and Health Maintenance in Healthy Humans

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Abstract

Aim: Animal studies have shown that Multi-Strain Probiotics (MSP) such as *Lactobacillus rhamnosus* LCRI77, *Bifidobacterium adolescentis* BA286, and *Pediococcus acidilactici* PA318, can inhibit fat formation and improve blood lipid levels, but the effects in humans are not known. This study compared the effects of MSP on blood pressure and health maintenance in healthy normal-weight and obese subjects.

Methods: Forty-six subjects, 20 to 50 years of age, were divided into a normal-weight group and a healthy obese group. The body mass indexes (BMIs) of 20 subjects in the normal-weight group ranged from 18.5 to 23.9 kg/m^2, while BMIs for the 26 subjects in the obesity group were all ≥ 27.0 kg/m^2. In a double-blind, random test, each subject consumed three capsules of an MSP formulation (2.23×10^{10} colony-forming units) each day after meals. All subjects maintained their normal activities of daily life, including diet and exercise patterns. Anthropometry and blood biochemical analysis were done, and the results of a heart and lung functional test were analyzed.

Results: All 46 subjects consumed the MSP for 6 weeks. The average weekly weight loss was 0.5%, and waist circumference, BMI, and systolic blood pressure were significantly lower among the normal-weight group ($P<0.05$). SGOT and SGPT levels were significantly lower among the obese group ($P<0.05$), while the serum calcium level was significantly increased ($P=0.016$). The serum sodium level was significantly increased in the normal-weight group. Plasma triglycerides, low-density lipoprotein cholesterol, total calories, and total calories/high-density lipoprotein-cholesterol ratios decreased, but plasma high-density lipoprotein-cholesterol was increased. There were no significant differences between the two groups. Serum glucose, thyroxine, renal function, and pulmonary function remained in the normal range for all subjects.

Conclusions: The results of this study showed that the MSP formula can improve weight loss, lower SBP, and maintain health for obese persons.

Keywords: Probiotic; *Lactobacillus*; Obesity; Systolic blood pressure; Health maintenance

Abbreviations: LAB: Lactic Acid Bacteria; HFC: High Fat-Control; HFL: High Fat-Control with Low-Dose LAB Product; HFM: High Fat-Control with Middle-Dose LAB product; HFH: High Fat-Control with High-Dose LAB Product; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide; TC: Total Cholesterol; TG: Triglyceride; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; AST: Aspartate Aminotransferase; ALT: Aspartate Aminotransferase.

Introduction

Obesity is now a global pandemic [1]. It is a serious health problem in developed countries, and is associated with major health risks such as Coronary Heart Disease (CHD), Cardiovascular Disease (CVD), diabetes, high blood pressure, metabolic syndrome, abnormal blood fats (including high levels of triglycerides and low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) [2]. Obesity also affects lifespan [3]. Between 2009 and 2010, the prevalence of obesity among adults in the U.S. was 35.5% for men and 35.8% for women [4].

Although the rate of obesity in Taiwan is lower than that of numerous European countries and the U.S., it is higher than that of most other Asian countries [5]. The Nutrition and Health Survey in Taiwan (NAHSIT) showed that the prevalence of overweight and obesity for adult men (>19 years of age) increased from 33.4% between 1993-1996 to 51.0% in 2005-2008, far higher than many other Asian countries [6]. In adult Taiwanese women, rates increased slightly, from 33.5% to 35.9% during the same time period [7]. In addition, comparisons of cardiovascular disease and metabolic syndrome markers showed that Taiwan's largest nutrition-related health problems are a rapidly rising obesity rate (including central obesity) and related metabolic syndrome. In particular, the number of cases of diabetes and hypertriglyceridemia are increasing [5].

The 2002 Taiwanese National Health Expenditure (NHE) showed that the treatment costs for obese metabolic syndrome was 2.9% of universal health insurance claims and about $16.2 billion [8]. This cost does not include medical illness and hospitalization or economic losses. The medical expense for obesity-related diseases was $7.85 billion in the U.S. in 1998, but by 2008 was $14.7 billion, an 87% increase over 10 years [9]. In Taiwan, obesity not only causes personal health problems, but also has a high economic cost.

Prevention and improvement of obesity is a very important public issue. However, prevention or amelioration of obesity by traditional approaches, such as diet, exercise, and drugs, is a long-term process, which requires great endurance and perseverance. The current common therapeutic approaches include medications, foods, and herbal health food, all of which are marketed as ways to achieve easy and rapid weight loss. However, their side effects and impact on health are often negative. Therefore, to maintain normal body weight and/or to achieve healthy weight loss should be an important public health goal [10]. This is also an important opportunity for medical industry and health care (health food, biotech food, medical supplies agencies). The Behavioral Risk Factor Surveillance System (BRFSS) data show that in 1996, 28.8% of men and 43.6% of women were using various products in an attempt to lose weight. In 1997, researchers in Australia also found that 22.9% of adults surveyed were trying to lose weight.

Probiotics were first used by Fuller in 2009 and are defined as live microbial food supplements that have a beneficial effect on human health through the gastrointestinal tract [11]. In 1974, Mann and Spoerry [12] reported that a Masai tribe in Africa that drank large amounts of fermented milk containing Lactobacillus lines had a low concentration of plasma cholesterol. Following this discovery, many researchers began to study the effect of Lactobacillus on cholesterol. Currently, the ability of probiotics to reduce serum cholesterol can be divided into three categories:

Bile salts and cholesterol bound to the co-precipitation [13]

Adsorption [14]

Inhibition of cholesterol synthesis [15,16].

However, it is unknown whether the effects of lactic acid can reduce body fat by lowering cholesterol, synthesis, or adsorption of fat.

The hypolipidemic and anti-obesity effects of lactic acid bacteria in animals [17,18] and humans [19] have become a hot topic in nutritional and food science research. Choi, et al. and Wang, et al. [18] also reported that the intake of Lactobacillus ferment reduced the amount of plasma triacylglycerol and the weight of epididymal and perirenal fat. The hypotriacylglycerolemic action of Lactobacillus ferment may be related to its effect on energy balance, and had a positive correlation between weight gain and triacylglycerolemia. The clinical study showed that the daily consumption of Lactobacillus ferment was associated with significantly reduced BMI, and an improved profile plasma triacylglycerols in humans [19,20].

Our previous animal studies showed that when male Wistar rats ate a high-dose compound of lactic acid bacteria (LAB) for 8 weeks, an increase in body fat could be inhibited. Furthermore, plasma triglyceride values and plasma LDL-C values were significantly lower than those of an experimental group and control group. In addition, when obese rats were fed a composition of lactic acid, cardiomyocytes injury was slowed significantly, and death-receptor-dependent and mitochondria-dependent apoptotic pathways were inhibited. In addition, the number of dead myocardial cells was inhibited among obese rats [18]. These same effects on the human body are unclear. Therefore, we designed a study to assess the effects of as MSP on blood pressure and healthy maintenance of weight loss among two groups of healthy humans.

Material and Methods

Participants

Forty-six adult patients participated in the study. The inclusion criteria were: age from 18-65 years; Body Mass Index (BMI) ≥ 18.5 kg/m^2; no history of cardiac, liver, kidney or endocrine disease or major diseases of other organ systems, or psychosis, or drug use. Subjects were excluded who had participated in a clinical pharmaceutical trial during the 3 months before the study or those with a history of metabolic disease (cancer, cardiovascular disease, acute or chronic liver dysfunction, diabetes-related complications, etc.), or a history of abnormal electrocardiograms, need for supplemental oxygen, or chronic respiratory disease. Other exclusions included long-term use of medications (anti-arrhythmics, cytotoxic agents), and significant laboratory abnormalities (SGOP, SGPT, and bilirubin) [6]. Exclusions also included a history of substance abuse (including alcohol), or mental illness in the past 2 years; pregnancy, congenital metabolic diseases, or lactic acid allergy [7]. The study was approved by the Institutional Review Board of Cheng Ching General Hospital (HP090019). Informed consent was obtained from all patients after the received a full explanation of the study's aim and protocol.

Interventions with multi-strain probiotics

Forty-six subjects were categorized into two groups according to BMI:

A non-obese group (20 people) with BMIs from 18.5-23.9 kg/m^2.

An obese group (26 people) with BMIs ≥ 27 kg/m^2.

All subjects were healthy and maintained their daily eating habits and did not limit their calorie intake. All subjects received the probiotic compound MSP (New Bellus Enterprise Co., Ltd., Tainan, Taiwan), in a daily dosage of 3 capsules after meals, then three times a day for 6 weeks. The MSP capsules (4g powders) contain seven bacterial species, including *Lactobacillus rhamnosus* LCRI77 (*L. rhamnosus*), *Bifidobacterium adolescentis* BA286 (*B. adolescentis*) and *Pediococcus acidilactici* PA318 (*P. acidilactici*). The total viable bacteria count is 2.23×10^{10} CFU/per capsule. Other Ingredients (0.5g powders) are Complex Enzyme, Xylooligosaccharides, Maltooligosaccharides, and Fibersol. No other treatment was prescribed for patients during the study period.

Data collection

The purpose of the study was explained to all subjects, and a signature or fingerprint was obtained for each subject before participation. All subjects completed a questionnaire at baseline, and data about nutritional intake and the impact of gastrointestinal symptoms were collected. The data were collected between May and September 2010. All research programs were conducted in accordance with the Declaration of Helsinki amended in 2000. Diet intake and nutrients analysis was using the E-kitchen Nutrition Data system software (E-kitchen Business Corp., Taipei, Taiwan).

Anthropometry measurements

Body fat percentage and weight were measured using a calibrated meter placed on a level surface. Body weight was recorded to the nearest 0.5 kg. Body height was measured at the start of the study, and recorded to the nearest 0.5 cm. The individual's waists were measured at a point midway between the lower rib margin and the iliac crest. Waist measurements were recorded to the nearest 0.5 cm. Hip circumference was measured around the top of the hips and buttocks at the widest point, and recorded to the nearest 0.5 cm. Waist-to-Hip Ratio (WHR) was the ratio of the circumference of the waist to that of the hips [21].

Blood analysis

Blood (10 mL) was drawn using venipuncture at the beginning and end of the experiment. Physical characteristics of blood were automatically analyzed by a blood analyzer (Cell-Dyn 3700, Abbott, USA). Fasting blood sugar, plasma Total Cholesterol (TC), HDL-C, LDL-C, Triglycerides (TG), Blood urea nitrogen (BUN), creatinine, uric acid, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), γ–GT, albumin and mineral (calcium, inorganic phosphorus, magnesium, chlorine, sodium and potassium) were measured (Kit, Randox Laboratoires Ltd, United Kingdom) with a Hitachi 7180 biochemistry analyzer (Hitachi, Tokyo, Japan).

Measuring blood pressure

Blood pressure is a measurement of the force on the walls of the arteries as the heart pumps blood through the body. Blood pressure was recorded at each visit using an Omron BP 742 monitor (Omron Healthcare, Inc., Bannockburn, IL, USA), after the subject sat quietly for 15 minutes.

Dietary information

The 24-hour diet recall was designed to investigate foods in the subject's regular diet, portions and methods of cooking the foods. Neutral but probing questions were used to help subjects recall all foods and beverages ingested in the past 24 hours. Food models, cutlery, and measuring tools were used to help the individual estimate portion size.

Statistical analysis

All data were analyzed in duplicate with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (Anova) with Duncan's multiple range tests was performed to compare any significant differences ($P<0.05$) in the variables between groups. Experimental data were presented as the mean ± Standard Deviations (SD) of the mean. Rank and frequency data were analyzed by a nonparametric test. Anthropometric values and gender were compared using the chi-square test and a 2-tailed independent t-test was used to compare dietary intake between the obese and non-obese groups.

Results

As shown in Table 1, mean weight gain among the obese group was 7.46% and 3.69% among the normal-weight group after 6 weeks of receiving the MSP supplement without limiting dietary intake. The difference between the two groups was not significant ($P>0.05$). After MSP intervention for 6 weeks, 85% of the men and women in the obese group had lost weight.

	BMI 18.5-23.9 (n=11)				**BMI ≥ 27 (n=15)**				**Comparing group (‡P-value)**
	Week 0 (A)	**Week 7 (B)**	**Difference (B-A)**	**†P-value**	**Week 0 (A)**	**Week 7 (B)**	**Difference (B-A)**	**†P-value**	
BW (kg)	56.30 ± 3.85	55.31 ± 4.01	-0.99 ± 0.49	0.003	81.61 ± 12.85	79.99 ± 12.80	-1.61 ± 1.22	0.000	0.125
BMI (kg/m^2)	22.40 ± 1.06	21.99 ± 1.16	-0.41 ± 0.17	0.003	30.89 ± 2.79	30.25 ± 2.86	-0.65 ± 0.54	0.000	0.177
Waist (cm)	74.55 ± 4.97	72.72 ± 4.70	-1.83 ± 1.98	0.024	91.33 ± 9.90	88.10 ± 9.61	-3.23 ± 3.26	0.003	0.197
Hip (cm)	88.41 ± 5.83	85.71 ± 5.41	-2.70 ± 3.35	0.033	107.06 ± 9.19	103.30 ± 7.61	-3.76 ± 3.50	0.004	0.427
WHR	0.844 ± 0.033	0.849 ± 0.037	0.005 ± 0.018	0.491	0.852 ± 0.024	0.851 ± 0.04	0.000 ± 0.025	0.937	0.515
BFP (%)	31.05 ± 1.98	30.30 ± 2.55	-0.75 ± 1.51	0.185	37.70 ± 9.35	37.68 ± 8.63	-0.02 ± 2.84	0.105	0.444
SBP (mmHg)	116.73 ± 16.42	112.00 ± 14.47	-4.73 ± 6.92	0.049	128.27 ± 15.35	121.60 ± 11.97	-6.67 ± 8.32	0.013	0.535
DBP (mmHg)	72.64 ± 11.60	70.64 ± 11.06	-2.00 ± 8.33	0.634	82.67 ± 12.74	82.80 ± 13.88	0.13 ± 9.58	0.753	0.560

MAP (mmHg)	87.36 ± 12.75	84.36 ± 10.68	-3.00 ± 7.50	0.342	97.87 ± 13.21	95.80 ± 12.19	-2.13 ± 7.77	0.200	0.778
Pulse rate (bpm)	77.45 ± 10.48	79.09 ± 10.67	1.64 ± 7.53	0.721	77.13 ± 8.85	74.20 ± 8.78	-2.93 ± 9.36	0.245	0.195
TC (mg/dL)	175.73 ± 47.93	174.64 ± 55.37	-1.09 ± 16.16	0.593	178.80 ± 28.93	178.00 ± 28.12	-0.80 ± 15.18	0.638	0.963
TG (mg/dL)	74.91 ± 33.05	65.09 ± 29.46	-9.82 ± 26.47	0.286	133.20 ± 97.20	136.27 ± 89.63	3.07 ± 65.09	0.410	0.543
HDL-C (mg/dL)	63.45 ± 8.02	64.73 ± 9.21	1.27 ± 5.66	0.350	49.87 ± 11.21	50.53 ± 11.31	0.67 ± 3.56	0.448	0.740
LDL-C (mg/dL)	101.00 ± 42.41	97.82 ± 49.43	-3.18 ± 13.75	0.286	109.13 ± 22.97	106.20 ± 24.78	-2.93 ± 12.66	0.363	0.962
TC/HDL-C ratio	2.81 ± 0.58	2.75 ± 0.70	-0.05 ± 0.26	0.475	3.76 ± 0.88	3.70 ± 0.87	-0.06 ± 0.39	0.562	0.968
BUN (mg/dL)	10.18 ± 2.44	11.27 ± 3.26	1.09 ± 3.65	0.348	13.07 ± 3.13	13.13 ± 3.66	0.07 ± 3.13	0.893	0.448
Uric acid (mg/dL)	4.85 ± 0.89	5.01 ± 1.02	0.16 ± 0.13	0.088	6.89 ± 1.88	7.24 ± 1.47	0.35 ± 1.14	0.078	0.286
FBS (mg/dl)	81.36 ± 7.49	81.45 ± 7.84	0.09 ± 7.08	0.789	83.08 ± 8.37	80.60 ± 6.77	-3.53 ± 5.55	0.323	0.125
SGOT (mg/dL)	18.09 ± 4.37	19.09 ± 7.15	1.00 ± 6.39	0.859	24.13 ± 12.79	19.73 ± 7.96	-4.40 ± 6.78	0.018	0.160
SGPT (mg/dL)	14.82 ± 6.65	14.36 ± 6.30	-0.45 ± 2.25	0.672	34.73 ± 33.83	27.27 ± 23.39	-7.47 ± 13.11	0.011	0.024
r-GT (mg/dL)	25.45 ± 17.90	25.36 ± 16.79	-0.09 ± 4.23	0.550	30.93 ± 17.15	31.53 ± 18.28	0.60 ± 4.07	0.721	0.834
ALP (mg/dL)	61.45 ± 13.92	62.64 ± 13.60	1.18 ± 4.60	0.440	70.47 ± 18.99	71.67 ± 21.55	1.20 ± 7.22	0.674	0.855
Albumin (g/dL)	4.41 ± 0.23	4.51 ± 0.15	0.10 ± 0.14	0.053	4.36 ± 0.23	4.41 ± 0.24	0.05 ± 0.16	0.321	0.220
Total protein (g/dL)	7.35 ± 0.38	7.55 ± 0.33	0.19 ± 0.37	0.106	7.40 ± 0.28	7.41 ± 0.23	0.01 ± 0.33	0.752	0.151
Globulin (g/dL)	2.95 ± 0.33	3.04 ± 0.37	0.09 ± 0.27	0.309	3.04 ± 0.28	3.00 ± 0.15	-0.04 ± 0.22	0.428	0.357
A/G ratio	1.58 ± 0.19	1.56 ± 0.25	-0.02 ± 0.15	0.732	1.51 ± 0.17	1.53 ± 0.12	0.02 ± 0.08	0.317	0.469
T4 (mg/dL)	7.29 ± 0.10	7.50 ± 1.23	0.21 ± 0.65	0.328	6.41 ± 0.89	6.59 ± 0.94	0.18 ± 0.53	0.156	0.917
Sodium (mg/dl)	140.64 ± 1.69	139.55 ± 1.57	-1.09 ± 1.38	0.046	140.27 ± 1.58	140.00 ± 1.31	-0.27 ± 1.67	0.576	0.175
Potassium (mg/dL)	4.24 ± 0.45	4.33 ± 0.53	0.09 ± 0.46	0.440	4.33 ± 0.23	4.37 ± 0.17	0.05 ± 0.24	0.799	0.581
Chloride (mg/dL)	102.45 ± 2.38	103.00 ± 1.18	0.55 ± 2.38	0.368	102.40 ± 1.59	102.60 ± 1.06	0.20 ± 1.93	0.749	0.713
Calcium (mg/dL)	8.76 ± 0.45	9.00 ± 0.31	0.24 ± 0.43	0.085	8.78 ± 0.35	9.05 ± 0.35	0.27 ± 0.37	0.016	0.958
Phosphorus (mg/dL)	3.48 ± 0.61	3.55 ± 0.56	0.07 ± 0.33	0.537	3.37 ± 0.30	3.53 ± 0.32	0.16 ± 0.32	0.083	0.514

Magnesium (mg/dL)	2.53 ± 0.22	2.47 ± 0.18	-0.05 ± 0.28	0.408	2.35 ± 0.19	2.34 ± 0.19	-0.01 ± 0.16	0.719	0.475
[a]Values are presented as mean ± SD. Non-parametric statistics. *P<0.05. **P<0.01. † Paired t-test result for both groups compared at weeks 0 and 7. ‡Independent test result for between-group difference comparison. TC/HDL-C ratios: Total Cholesterol (TC)/High-Density Lipoprotein Cholesterol (HDL-C) ratio was used to predict ischemic heart disease risk. BFP: Body Fat Percentages; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Pressure; BUN: Blood Urea Nitrogen; A/G ratio: Albumin-Globulin in Ratio. ALP: Alkaline Phosphomonoesterase; TG: Fasting Plasma Triglyceride								

Table 1: Effects of MSP clinical characteristics on 0.5% weight loss of the subjects (n=26)[a].

Moreover, as can be observed from the ratio of weight lost>0.5% was 56.52%, of weight lost ≥ 0.2% was 63.04%. The ratio of weight unchanged and weight increased was 6.52% and 30.43%, respectively. One subject lost 5 kg; 19 subjects lost ≥ 1 kg; and 26 subjects lost ≥ 0.5%. A weight loss ≥ 0.5% meant an average reduction of 0.165 kg per week for the normal subjects, and 0.268 kg per week in the obese subjects. The weight, BMI, and waist and hip circumference of all subjects after taking the MSP supplement for 6 weeks were significantly lower (P<0.005), but the difference between groups was not significant (P>0.005). In addition, there were no significant differences in waist-hip ratio and body fat percentages of subjects (P>0.005).

After intervention with MSP for 6 weeks (Table 1), Systolic Blood Pressure (SBP) levels were significantly reduced: 4.73 ± 6.92 mm Hg and 6.67 ± 8.32 mm Hg for the normal group and the obese group, respectively (P<0.001). The obese group lost up to 0.5% of their pre-study weight. Diastolic blood pressure was reduced by 2.00 ± 8.33 mmHg for the normal-weight group. Mean Arterial Pressure (MAP) decreased 3.00 ± 7.50 mmHg and 2.13 ± 7.77 mmHg for the normal-weight group and the obese group, respectively. Pulse rate increased 1.64 ± 7.53 Beats Per Minute (BPM) and decreased 2.93 ± 9.36 BPM for the normal-weight group and the obese group, but the difference was not significant (P>0.001).

Plasma Fasting Blood Sugar (FBS) levels were reduced 3.53 ± 5.55 mg/dL for the obese group, and plasma TG levels fell by 9.82 ± 26.47 mg/dL and 0.933 ± 62.27 mg/dL for the normal group and the obese group, respectively; the difference was not significant (P>0.05). Plasma HDL-C levels (63.45 ± 8.02 mg/dL and 64.73 ± 9.21 mg/dL) of the normal-weight group were higher than those of the obese group (49.87 ± 11.21 mg/dL and 50.53 ± 11.31mg/dL), and was an increasing trend (1.27 ± 5.66 mg/dL and 0.67 ± 3.56 mg/dL). TC, LDL-C and the arteriosclerosis index decreased (3.18 ± 13.75 mg/dL, 1.09 ± 16.16 mg/dL, 0.05 ± 0.26, and 2.93 ± 12.66 mg/dL, 0.80 ± 15.18 mg/dL, 0.06 ± 0.39) between the two groups, but no significant difference was noted (P>0.05), as shown in Table 1.

Serum Urea Nitrogen (BUN), creatinine, and uric acid values of the weight lost among 0.5% of subjects were not significantly different between the two groups before and after the test, and all data were within the normal range (Table 1). The SGOT and SGPT (Table 1) were significantly decreased, by 4.40 ± 6.78 U/L in the obese subjects (P=0.018) and 7.47 ± 13.11 U/L (P=0.011) in the normal-weight group. The SGPT and r-GT were slightly decreased in the normal-weight group (0.45 ± 2.25 U/L, 0.09 ± 4.23 mg/dL). Alkaline Phosphatase (ALP), albumin, total protein, and the T4 thyroid indexes of the two groups were slightly increased, but there were no significant differences. In addition, serum calcium, potassium, chloride, and phosphorus levels of the two groups had an upward trend. There was a significant difference in serum calcium levels for the obese and normal-weight groups (P<0.05). Serum sodium levels were decreased in both groups, and there were significant differences between the normal-weight and obese groups (P<0.05).

Pearson correlation coefficient between change of anthropometry and its related factor analysis is shown in Table 2. Weight loss was associated with BW, BMI, SBP, DBP, MAP, FBS, TC/HDL, ALP, potassium and globulin, but was negatively correlated with serum albumin and the A/G ratio. There was a significant positive correlation between weight loss and BW, BMI, SBP, DBP, MAP, FBS, TC/HDL, ALP, potassium, and globulin. However, it was significantly negatively correlated with height, IBW, HDL-C, creatinine, sodium, chloride, magnesium, albumin and A/G ratio. Body weight loss was significantly positively correlated with height, weight, IBW, BMI, SBP, DBP, MAP, FBS, TG, TC/HDL, SGOT, SGPT, ALP, creatinine, uric acid, potassium, calcium and dietary fat. There was a significant negative correlation with HDL-C expression.

	Waist	Change of body weight	Change of body fat
Age	-0.13704	-0.03595	-0.04636
Body height	0.25323*	0.07819	-0.38598*
Body weight	0.83511*	0.11466	0.41127*
IBW	0.25842*	0.07535	-0.39055*
BMI	0.84391*	0.09732	0.74734*
Pulses rate	0.00990	0.05660	0.16515*
SBP	0.46397*	0.13102	0.19986*
DBP	0.50617*	0.12141	0.18693*

MAP	0.52042*	0.13440	0.20337*
FBS	0.28018*	-0.00544	0.20499*
TC	0.00059	-0.05773	-0.08753
TG	0.39593*	-0.02006	-0.01670
HDL-C	-0.46827*	0.04489	-0.26273*
LDL-C	0.09861	-0.07042	0.00686
TC/HDL	0.47190*	-0.08304	0.18220*
SGOT	0.23283*	-0.00083	0.08625
SGPT	0.35546*	-0.00757	0.09344
ALP	0.24282*	-0.02861	0.32382*
BUN	0.11006	-0.10606	-0.06906
Creatinine	0.18697*	-0.06824	-0.36937*
Uric acid	0.43137*	-0.00921	0.06752
Sodium	0.00957	-0.09403	-0.24451*
Potassium	0.16741*	0.20081*	0.16891*
Chloride	-0.08723	-0.06831	-0.21144*
Calcium	0.14918*	-0.09036	-0.07792
Phosphorus	-0.03199	0.09436	-0.04140
Magnesium	-0.10536	0.06419	-0.16113*
Albumin	-0.11859	-0.15862*	-0.40482*
Total protein	-0.06776	0.01761	-0.06662
Globulin	0.00968	0.13963	0.22580*
A/G ratio	-0.07137	-0.18682*	-0.35355*
Dietary calories	0.01070	-0.07379	-0.19028*
Dietary protein	-0.03971	-0.03549	0.01818
Dietary fat	0.20089	0.07630	-0.06578
Dietary carbohydrate	-0.17510	-0.05098	0.05508

[a]Pearson correlation coefficient. *P<0.05. **P<0.01. TC/HDL-C ratios: Total Cholesterol (TC)/High-Density Lipoprotein Cholesterol (HDL-C) ratio was used to predict ischemic heart disease risk. BFP: Body Fat Percentages; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Pressure; BUN: Blood Urea Nitrogen; A/G ratio: Albumin-Globulin in Ratio. ALP: Alkaline Phosphomonoesterase; TG: Fasting Plasma Triglyceride

Table 2: Pearson correlation coefficient between change of anthropometry and body position related factor analysis.

This regression analysis was further used to predict the impact of change in anthropometry factors. Table 3 shows the predictive factors for weight changes, as follows: MAP, HDL-C, albumin, total protein, potassium, calcium and phosphorus (DF=7, F=4.21, P<0.0001). That is, weight changes will exceed 0.5%, due to changes in these factors to the conditions in Table 3 after consumption of MSP. Table 4 shows the predictive factors for body fat changes sequence as follows: age, height, weight, BMI, pulse rate, TC, LDL-C, TC/HDL, creatinine, calcium, and dietary fat (DF=11; F=208.08; P<0.0001). That is, weight changes will exceed 0.5% due to changes in these factors (Table 4) after consumption of MSP. Table 5 lists the predictive factors for waist changes sequence, as follows: body fat change, height, IBW, BMI, SBP, TC, HDL-C, TC/HDL, BUN, creatinine, magnesium, dietary calories and dietary carbohydrate (DF=13, F=45.04, P<0.0001). That is, weight changes will exceed 0.5%, due to changes in these factors in the conditions listed in Table 5, after consumption of MSP.

Summary of the stepwise multiple regression analysis examining the effect of body weight, age, body height and foot length on postural parameters with and without vision (n=46).

	Parameter estimate	Standard error	Type II SS	F-value	Pr>F
Intercept	568.90161	237.78452	8.69496	5.72	0.0179
MAP	0.02905	0.00910	15.48507	10.19	0.0017
HDL-C	0.01738	0.00790	7.34805	4.84	0.0293
Albumin	-1.49360	0.60556	9.24082	6.08	0.0147
Total protein	0.69504	0.32822	6.81149	4.48	0.0358
Potassium	1.15585	0.33346	18.25010	12.01	0.0007
Calcium	-0.97825	0.40506	8.85996	5.83	0.0169
Phosphorous	0.48597	0.23130	6.70534	4.41	0.0372
Variable smg Removed: R-Square=0.2871 and C(p)=-7.9018					
Source	DF	Sum of squares	Mean square	F-value	Pr>F
Model	7	96.02342	6.40156	4.21	<0.0001
Error	165	238.48456	1.51901	-	-
Corrected Total	172	334.50798	-	-	-

Table 3: Regression analysis of predict factors of body weight change.

Discussion

Obesity is associated with serious medical complications that impair quality of life [2] and impact lifespan [3]. The aim of this study was to evaluate the effects of the lactobacilli mixtures (MSP) on health management of healthy obese subjects and normal-weight subjects. We showed that 6 weeks after the subjects in the intervention group received MSP, dietary intake (total calories, protein and lipid) had also increased, but the differences were not significant.

Previous animal studies have shown that MSP increased food and water intake, and feeding efficiency in experimental animals, but these were not significant differences. Interestingly, at the end of the experiment, food intake was significantly increased (2.62%) for the HFH group compared to the NC and HFC groups ($P<0.05$). Simultaneously, HFH group water intake increased 4.12%, and feed efficiency decreased 12.25%; there were no significant differences compared to the NC and HFC groups. But, specifically, HFH delayed weight gain, and reduced the formation of fat surrounding the epididymis and adrenals [18]. The present study was further proof that MSP given to normal-weight or obese subjects will significantly reduce weight, BMI, and waist and hip circumference, respectively (Table 2). Therefore, this study MSP product (Pro bioS-23) theoretically might have to adjust the weight of the role.

Variable	Parameter estimate	Standard error	Type II SS	F-value	Pr>F
Intercept					
Age	-0.30916	0.05391	72.51901	32.89	<.0001
Body height	0.72851	0.12251	77.97406	35.36	<.0001
Body weight	-0.40440	0.12899	21.67594	9.83	0.0021
BMI	2.54724	0.33727	125.78447	57.04	<.0001
Pulse rate	0.03705	0.01224	20.21950	9.17	0.0029
Total Cholesterol	-0.04743	0.01295	29.57595	13.41	0.0003
LDL-C	0.06437	0.01630	34.40650	15.60	0.0001
TC/HDL	-0.72610	0.23660	20.76892	9.42	0.0025
Creatinine	-5.54315	1.22467	45.17696	20.49	<.0001
Calcium	0.84519	0.43150	8.46019	3.84	0.0520
Dietary Fat	-0.02949	0.01530	8.18771	3.71	0.0558
Variable waist Removed: R-Square=0.9627 and C(p)=2.4362					
Source	DF	Sum of squares	Mean square	F-value	Pr>F
Model	11	8718.09786	458.84726	208.08	<.0001
Error	161	337.39266	2.20518	-	-
Corrected Total	172	9055.49052	-	-	-

Table 4: Regression analysis of predictive factors of body fat change (n=46).

Variable	Parameter estimate	Standard error	Type II SS	F Value	Pr>F
Intercept	912.73427	233.37551	297.00707	15.30	0.0001
Body fat change	0.42800	0.15753	143.34010	7.38	0.0074
Body height	-7.20450	2.16078	215.86087	11.12	0.0011
Ideal body weight	10.89726	3.07452	243.93210	12.56	0.0005
BMI	1.74824	0.27328	794.62582	40.92	<.0001
SBP	0.04991	0.02684	67.15498	3.46	0.0649
Total cholesterol	-0.06820	0.02655	128.13313	6.60	0.0112
HDL-C	0.32274	0.08511	279.20500	14.38	0.0002

TC/HDL	4.19749	1.40841	172.46722	8.88	0.0034
Blood urine nitrogen	-0.45428	0.14988	178.37287	9.19	0.0029
Creatinine	9.37570	3.93164	110.42006	5.69	0.0183
Magnesium	5.31156	1.84892	160.24945	8.25	0.0047
Dietary calories	-0.00224	0.00067367	215.56833	11.10	0.0011
Dietary carbohydrate	-0.07193	0.04001	62.76758	3.23	0.0742
Variable waist removed: R-Square=0.9627 and C(p)=2.4362, F-value=45.04, P<0.0001					

Table 5: Regression analysis of predictive factors of waist circumference change (n=46).

According to results from the Taiwan Survey on Hypertension, Hyperglycemia, and Hyperlipidemia (TwSHHH II) in 2002 and 2007, the prevalence of obesity and blood pressure was positively correlated [5]. The study results by Selmer et al. further illustrate that reducing systolic blood pressure by 4%, or by 2 mm Hg, can reduce coronary deaths, stroke mortality by 6%, and reduce 5% of all deaths [22]. Reducing diastolic blood pressure by 5 mm Hg could lower coronary artery mortality by 9%, coronary artery mortality, by 14%, and could eliminate 7% of all deaths from stroke in the U.S. among persons 45-64 years of age. The results of this study showed that after 6 weeks of intervention, there was a mean weight loss of 0.5% among the normal-weight group, and the systolic blood pressure of the healthy obese group was also significantly lower (Table 1). It follows that in the present study; the MSP product improved obese systolic blood pressure, and thus may have a preventive effect on coronary artery disease and stroke deaths.

In cellular tests, MSP has been shown to significantly improve BSH activity and bile acid de-conjugation ability, and can significantly lower cholesterol and TG. In animals, use of MSP significantly lowered cholesterol, LDL-C, the total concentration of serum triglycerides or high cholesterol acid-reactive substances with a high-fat hamster liver, and thiobarbituric-acid-fed hamsters and a high cholesterol diet. The results show that the effects of the MSP were greater for reducing the risk of cardiovascular disease [23]. Other studies have shown that Lactobacillus and *Bifidobacterium longum* [24], including L. acidophilus have a significant cholesterol-lowering effect in rats and humans [25].

Our results showed that the subject's serum TG, TC, LDL-C and HDL/TC values showed a decreasing trend, while increasing HDL-C values in the intervention 6 weeks after taking MSP products. The reduction of serum TC and TG while increasing HDL-C has reduced risk of coronary heart disease risk [26]. In 1999, James et al. used yogurt containing *L. acidophilus* L1, giving adults daily 200 mL [27]. After the subjects continued drinking the yogurt drink, after 4 weeks the authors found that *L. acidophilus* L1, having high cholesterol, reduced the body's function. In 1987 survey, each 1% reduction in blood cholesterol levels, or about 2-3%, reduced the risk of coronary heart disease [28]. Thus, frequently eating foods containing *L. acidophilus* may be able to reduce the risk of coronary heart disease by 6% to 10%.

Lactic acid significantly decreased tert-butyl hydroperoxide (t-BHP)-induced liver injury in HepG2 cells. This exerted a protective effect by decreasing the risk of accumulated Reactive Oxygen Species (ROS) and by reactivating antioxidant enzymes [29]. Experiments in animal models have shown that LAB can effectively improve liver function [30,31] and lead to a significant decrease in human serum liver function. The reduction has been approximately 12% to 25%.32 The results of the present study show that MSP contributes to a significant decrease in these hepatic indicators, especially when the values among the healthy obesity subjects fell within the moderate ranges (SGOT 0-40 mg/dL, and SGPT 0-37 mg/dL). The results showed a nearly 19% decrease in these hepatic indictors in the healthy obese group ($P<0.05$). However, there was a slight decrease in SGPT in the normal-weight group, but this was not significant ($P>0.05$). Thus, long-term consumption of health products such as MSP should have a protective effect on liver function among obese persons.

The results of this study also demonstrated that adding MSP to the diet does not affect fasting blood glucose and renal function (including BUN, creatinine and uric acid), and remain in the normal range. Similar results were found in animal experiments: no significant differences were seen in fasting blood glucose levels in obese and normal-weight mice after the animals consumed LAB [32].

Surprisingly, our research showed that tetraiodothyronine (T4) values slightly increased (0.21 ± 0.65 mg/dL, or 2.88% and 0.18 ± 0.53 mg/dL, or 2.81%, with reduction of body weight of 0.5% in the normal-weight group and healthy obese subjects (Table 1). T4 is the thyroid secretion that regulates metabolism and growth rate, as well as mediating other body systems. T4 is the mediator of pulse rate, increases in blood pressure, vasodilation, and increases in body temperature. Thus, it may increase energy consumption and promote appetite. In addition, the thyroid gland releases calcitonin, another hormone, to regulate blood calcium concentration. When blood calcium levels are too high, the thyroid secretes calcitonin. Calcitonin then draws calcium ions into bone; thus, it is an important mechanism for the prevention of osteoporosis [33].

Our major discovery was the significant increase in serum calcium values after supplementary MSP in the obese group (while still within the normal range). Therefore, MSP supplements should be continued, which may help prevent osteoporosis.

Previous studies have shown that unhealthy diet and lifestyle, including lack of exercise are important factors leading to obesity. Obesity can affect physiological metabolism, including blood pressure, blood lipid levels, and blood sugar levels. Factors that affected weight loss are shown in Table 3. When MAP, total protein, serum potassium, and phosphorus increased, plasma HDL-C, albumin, and serum calcium were reduced (by a coefficient of variation of 2 values). In such

a case, the weight change will be>0.5%. The coefficient for predicting weight change was as follows:

Intercept=568.90161+(MAP × 0.02905)-(HDL-C × 0.01738)-(albumin × 1.49360)+(total protein × 0.69504)+(potassium × 1.15585)-(calcium × 0.97825)+(phosphorus × 0.48597).

This result showed that MSP supplement increased T4 slightly in serum, which may increase the metabolic rate, and therefore also increase the pulse rate of those who lose weight. Therefore, if obese persons receive continuous supplementation of MSP LAB products, this may lower body weight, and reduce waist circumference and blood pressure. This may also lead to further health maintenance and prevention of cardiovascular disease.

This study had some limitations. We assessed blood pressure, anthropometry, and blood composition, the most important elements for maintaining health for healthy obese persons. This type of assessment is based on the clinical importance of change; however, it might not detect small changes. Therefore, in future studies it would be better to apply a more comprehensive evaluation of symptoms [34]. Furthermore, healthy maintenance involves evaluating blood pressure, anthropometry, and blood composition of the patient in the preceding 30 days. Thus, 6 weeks was not an appropriate interval for expecting change in health maintenance, and longer follow-up was needed to clearly maintain health.

Conclusion

This study demonstrated that MSP interventions can reduce body weight by 56% in subjects with unrestricted calorie intake, and have a role in lowering blood pressure and maintaining health. Therefore, an MSP intervention can help obese persons maintain a healthy weight. Future studies are needed to further explain this effect of energy balance and blood pressure mechanisms.

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